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Quantitative Analysis of Micro-Structure in Meat Emulsions from Grating-Based Multimodal X-Ray Tomography

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ABSTRACT

Using novel X-ray techniques, based on grating-interferometry, new imaging modalities can be obtained simultaneously with absorption computed tomography (CT). These modalities, called phase contrast and dark field imaging, measure the electron density and the diffusion length of the sample. Enhanced contrast capabilities of this X-ray technique makes studies on materials with similar attenuation properties possible. In this paper the focus is set on processing grating-based X-ray tomograms of meat emulsions to quantitatively measure micro-structural changes due to heat treatment. The emulsion samples were imaged both in a raw and cooked state. Additionally, different fat types were used in the emulsions in order to compare micro-structural differences when either pork fat or sunflower oil was used. From the reconstructed tomograms the different ingredients in the emulsions were segmented using a multivariate segmentation method. From this, a quantitative analysis was performed between the different samples, determining properties such as percentage object volumes and cooking loss. Additionally, the porosity, degree of anisotropy and average structure thickness of the protein networks were determined. Analyzing the multivariate dataset instead of the single univariate absorption modality gave superior segmentation results. The quantitative analysis of the micro-structure gives insight to how both heat treatment, and the use of different lipid types, affect the final protein network.

1 Introduction

In recent years, X-ray micro-tomography (μ CT) has been an emerging technique for investigating the internal structure of food products [Frisullo et al. 2009, Herremans et al. 2011, Laverse et al. 2012]. The capability of imaging three dimensional structures has given rise to determination of structure parameters such as the porosity of matrices, percent object volumes (POV) and degree of anisotropy of food networks. In most of these studies, focus is set on analyzing a binary representation of the sample in question, for instance fat and protein in meat products and air voids in bread products. This limitation is mainly due to the contrast capabilities of conventional absorption X-ray techniques, where studying samples containing ingredients with similar attenuation properties is difficult. With emerging X-ray techniques based on grating interferometers, higher contrast levels can be obtained between ingredients not distinguishable from one another in absorption X-ray tomography. The focus of this study is to quantitatively analyze the micro-structure of meat emulsions and the effect that both different lipids (animal fat and vegetable oil) and heat treatment have on the protein network. The samples were imaged using the novel grating-based X-ray technique, to obtain contrast between the different ingredients. With this method, three data volumes were obtained simultaneously, where the absorptive-, refractive- and scattering properties of the samples are measured. Additionally, a multivariate contextual segmentation was implemented, combining Gaussian mixture models and a graph cut segmentation method [Boykov et al. 2001]. Several quantitative parameters were then determined in order to analyze the effects the different lipid types and heat treatment have on the protein structure. These parameters include the POVs, porosity of the protein structure, water loss due to heat treatment and the average protein network thickness. Although these parameters are presented in this paper, the main focus is set on the segmentation itself and the possibilities of exploiting such a segmentation rather than performing a thorough analysis of the underlying interactions between the protein network and different fat types.

2 Material and Methods

2.1 X-ray modalities

In Fig. 1 the three types of physical interactions - absorption, refraction and scattering - used as imaging modalities in grating-based interferometry are illustrated. The effect on an incoming Gaussian shaped beam profile (black) is depicted when elements with different physical properties are measured. The profiles shown in color represent what is recorded when a material is present. In green, the effect of an absorptive material is shown to attenuate the beam, while in blue, the effect of a refractive material is seen to cause a transverse shift in the position of the beam profile. Lastly, the small-angle scattering from a material with ordered micro-structures causes the beam profile, here shown in red, to broaden. By separating the attenuation, transverse shift and broadening of the beam, it is thus possible to measure three complementary imaging modalities. This can be done by grating-based imaging (GBI), which relies on an X-ray interferometer, consisting of periodic gratings for measurements. For further details, the reader is referred to [Bech et al. 2010, Pfeiffer 2012].

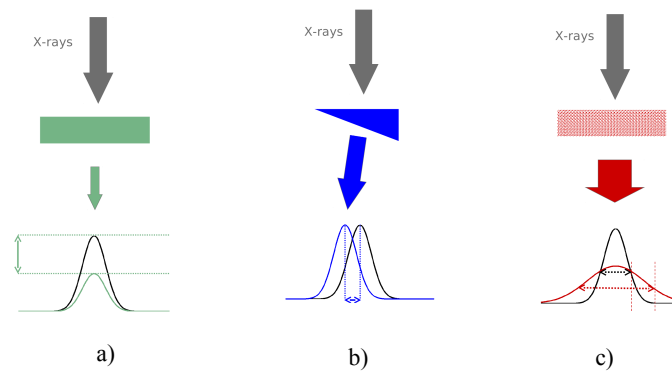


Fig. 1 The incoming X-ray beam changes when a sample is present. a) shows the effect from an absorptive material on the beam, b) a refractive material and c) a material with a homogeneous distribution of micro-structures.

2.2 Meat Emulsions Samples

The meat emulsions were prepared in batches of 1Kg in a food processor (CombiMax 600, Braun, Germany). Thawed meat (480 g), potato starch (5 g), curing salt (NaCl with 0.6% nitrite) (17 g) and crushed ice (248 g) were comminuted at highest speed for 2 minutes. The temperature at this point was 1°C in all batters. After addition of 250 g of either hand chopped cubes of lard or sunflower oil the batter was comminuted for 2 minutes. The temperature was measured (approx. 12°C) and comminuting was continued for 1 minute. End temperature was 14°C. For both the animal fat and sunflower oil batches, a portion was put in a sample container. The samples were then centrifuged at 5000g for 10 min, and had the lid closed under the surface of degassed PBS. The PBS-buffer was degassed to avoid bubble formation during scanning. For the cooking of the samples, a 200 mL glass of water was heated in a microwave up to boiling point. The sample in the container was then immediately placed in the water and left to stand for 15 minutes for the animal fat sample and 10 minutes for the sunflower oil sample. Both samples were then placed in a cold water bath, 10 minutes for the sunflower oil sample and 15 minutes for the animal lard sample.

2.3 Tomography Measurements

Absorption, phase-contrast and dark-field CT scans of both the raw and cooked meat emulsions were obtained at the TOMCAT beamline at the Swiss Light Source, Paul Scherrer Institut (PSI), Villigen, Switzerland. The setup is described in detail in [McDonald et al. 2009]. For this study the energy was set to 25 keV, and the third fractional Talbot distance used. The full volumes obtained were 1720x1720x513 voxels, with an effective pixel size at sample of 7.4 µm.

2.4 Multivariate Contextual Segmentation

The grating based imaging results in three data volumes with voxel correspondance. Multivariate methods are therefore possible for image analysis. A two step segmentation method was implemented, which considers both the spectral and spatial context of the data. First, the voxels are considered as stochastic variables where each voxel represents an observation $\mathbf{x}_i = (x_{i,1}, x_{i,2}, x_{i,3})^T$, $i=1, \dots, N$, where N is the number of voxels and $(x_{i,1}, x_{i,2}, x_{i,3})$ represent the absorptive-, refractive-, and scatter intensities of the i th voxel, respectively. The data is then modelled as a mixture of multivariate Gaussian distributions using an expectation-maximization (EM) algorithm [Hastie et al. 2009]. From this, the a priori multivariate distributions of the ingredients in the sample are obtained as

$$\phi(\mathbf{x}|\boldsymbol{\mu}_j, \boldsymbol{\Sigma}_j) = \frac{1}{(2\pi)^{3/2} |\boldsymbol{\Sigma}_j|^{1/2}} \exp\left(-\frac{1}{2}(\mathbf{x} - \boldsymbol{\mu}_j)^T \boldsymbol{\Sigma}_j^{-1} (\mathbf{x} - \boldsymbol{\mu}_j)\right) \quad (1)$$

where $\boldsymbol{\mu}_j = (\mu_{j,1}, \mu_{j,2}, \mu_{j,3})$ is the multivariate mean value for each distribution j and $\boldsymbol{\Sigma}_j$ is the corresponding full covariance matrix. Considering a voxel-wise classification function, each voxel is classified as a specific ingredient by finding the maximum probability of belonging to one of the multivariate Gaussian distributions given by Eq. 1. However, such a classification does not consider the spatial context of the data. Therefore the next step of the segmentation is to model the data as a Markov Random Field (MRF). MRFs are well known within image segmentation, and can be used to model the spatial relations between voxels. The smoothness of the resulting segmentation can be controlled with parameters that steer the allowed interaction in the three spatial directions. Using graph cuts, the energy function (given by Eq. 1) is minimized efficiently using approximation algorithms as described in [Boykov et al. 2001]. In addition, the alpha expansion algorithm [Boykov et al. 2001] was implemented to extend the MRF to multiple labels.

3 Results

A single slice from each tomographic reconstruction of the emulsion samples is shown in Fig. 2. Here, the dark ring is the plastic container the emulsions are placed in. The animal lard is seen as darker globules in the emulsions and the slightly lighter regions in the emulsions with sunflower oil are pure protein that have not been mixed with the rest of the emulsion. In both the absorption and phase contrast slices, it can be seen that the protein network has a darker intensity for the sunflower oil emulsions than the emulsions mixed with animal lard. This is due to the mixture of oil and meat, giving the overall protein network a lower intensity than pure protein. It is apparent that the phase contrast modality results in the highest contrast between the different ingredients. Although the dark field modality seems mainly to consist of noise, further inspection shows that high contrasts can be seen at edges where different ingredients meet.

Fig. 3 c) shows the result from the multivariate contextual segmentation for the cooked animal lard sample. In comparison, the result from a normal univariate threshold segmentation applied to both the absorption and phase contrast modalities are illustrated in Fig. 3 a) and b), respectively. The threshold values were determined by simply analyzing the valleys, peaks and curvatures of the histograms for each modality. For all segmentations, the red regions represent the protein network, the white globules are animal lard, the gray region is the sample container and the blue regions are water. When inspecting the normal threshold for the absorption modality in Fig. 3 a), the most obvious segmentation fault is that the low contrast between the container and fat on one hand, and the protein and water on the other, result in an erroneous classification. Any post-quantitative analysis would be greatly faulted and give a wrong interpretation of the actual sample. The high contrast between the ingredients in the phase contrast modality results in a better segmentation. Despite the high contrast, the fat and container are not completely separated in the segmentation. The multivariate contextual segmentation considers the three modalities as a mixture of multivariate Gaussians, and in a single segmentation the classification between the different ingredients is superior to the univariate threshold method. Also, considering all modalities simultaneously reveal a new phase in the protein network, illustrated by the brighter red regions surrounding the fat globules.

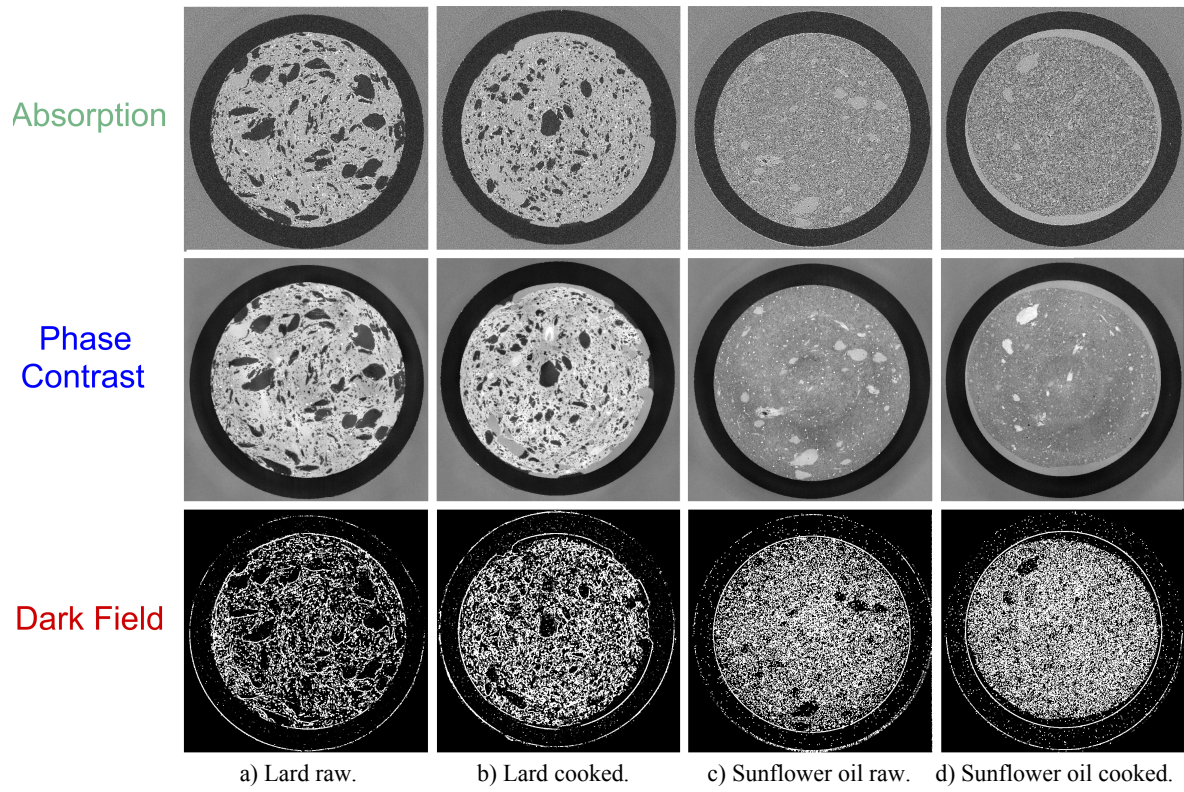


Fig. 2 Transverse slices from each sample and every modality. The phase-contrast modality results in the highest contrast between the different materials. Also, noise is more obvious in the absorption and dark field signal. The images are histogram equalized for better visualization.

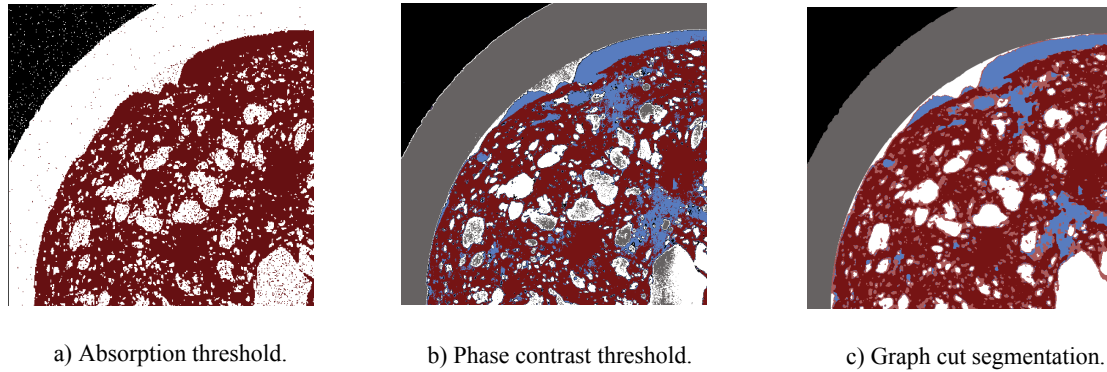


Fig. 3 The result from a univariate threshold segmentation for a) the absorption modality and b) the phase contrast modality. The result from the graph cut segmentation is shown in c).

Fig. 4 illustrates the results from the multivariate contextual segmentation for each sample. The color labels are explained at the top of Fig. 4. It should be noted that due to resolution limitations, the sunflower oil and moisture added to the emulsion mixture can not be separated from the protein network. These ingredients are therefore segmented as a single protein mixture phase. Considering the protein networks of the cooked samples, the use of sunflower oil seemingly results in a more homogeneous network after heat treatment. Given the obtained segmentations, a quantitative analysis of the emulsion structures was performed. Table 1 gives the percentage object volumes (POV) for each ingredient in the sample container. For the lard sample, the protein mixture volume decreases by 14.5% due to water loss after heat treatment. The protein mixture volume in the sunflower oil sample only decreases by 8.2%. The salt particles are completely dissolved after heat treatment.

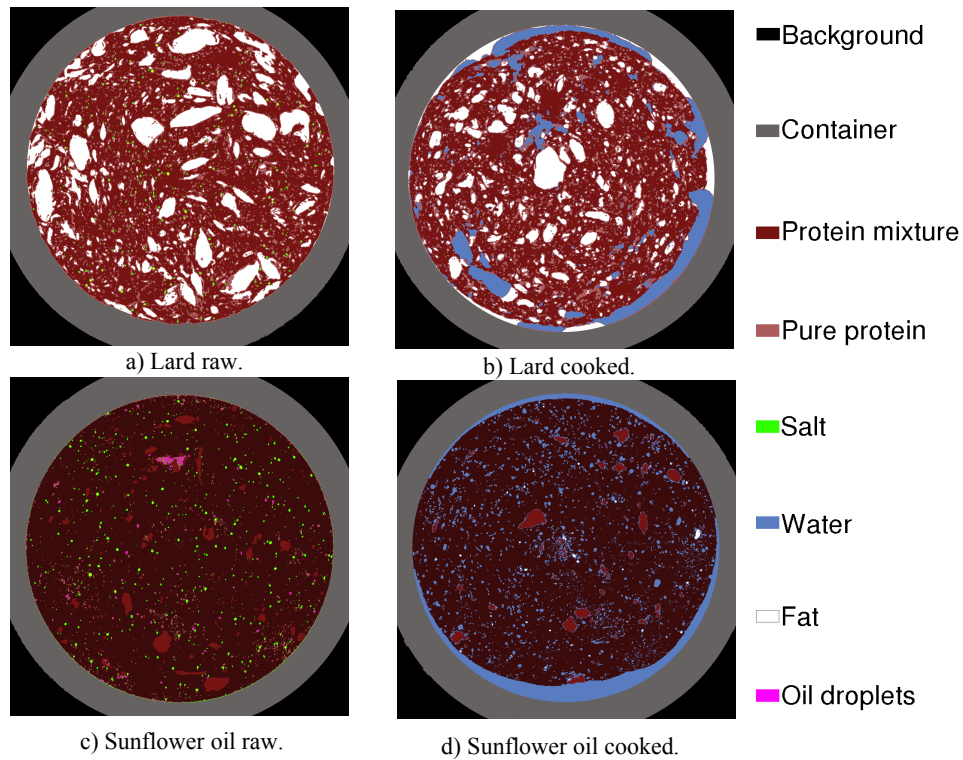


Fig. 4 A slice from each of the segmented volumes obtained from the multivariate contextual segmentation.

Table 1 Percent object volumes for the ingredients in the emulsion samples. The protein phases include both the sunflower oil and moisture which could not be separated due to resolution limitations.

Parameter	Lard raw	Lard cooked	Sunflower oil raw	Sunflower oil cooked
Protein (%)	73.9	59.4	98.1	89.9
Water (%)	-	15.6	-	9.6
Fat (%)	25.3	25.0	-	-
Salt (%)	0.8	-	1.7	-
Oil droplets (%)	-	-	0.2	0.5

Several quantitative parameters were also extracted, see Table 2. Although the cooking loss for the sunflower oil sample is greater, the POV of the entire water population is lower than for the lard sample. The water populations within the sunflower oil emulsion have a smaller average volume, which contributes to the resulting homogeneity. This is also highlighted by the degree of anisotropy, which is scaled such that a perfectly isotropic cylinder has the value of 0, and the most anisotropic structure (the cooked lard sample) has the value 1. The average protein structure thickness further underlines the differences in the protein structures.

Table 2 Quantitative parameters for the emulsion samples. Cooking loss gives the percentage of water separated from the emulsion, porosity is the pore volume divided by the total volume of the emulsion, anisotropy gives the degree of 3D symmetry in the emulsion structure, and the structure thickness is the average of the local thickness of the protein network.

Parameter	Lard raw	Lard cooked	Sunflower oil raw	Sunflower oil cooked
Mean fat volume (μm^3)	$1.19 \cdot 10^6$	$9.80 \cdot 10^5$	-	-
Mean water volume (μm^3)	-	$1.68 \cdot 10^6$	-	$3.92 \cdot 10^5$
Cooking loss (%)	-	7.4	-	8.3
Porosity (%)	26.1	35.6	6.4	7.1
Anisotropy (-)	0.45	1	0.12	0.16
Structure thickness (μm)	47.36	33.3	100.64	68.08

4 Discussion

Considering the POVs, the segmented raw protein phase for the lard sample is found to be 73.9%, salt is 0.8% and fat 25.3%. When comparing to the weighted ingredients (480 g meat, 5 g starch, 1.7 g salt, 248 g ice, 250 g fat) we note that the combined weighted percentage of the meat, starch and ice amounts to 73.3 % of the 1 kg batch. Since these ingredients are all segmented as the single protein mixture phase, the results fit well. The same goes for the sunflower oil sample. Here, the found POV for the segmented protein and sunflower oil mixture is 98.3% and the POV for salt is 1.7%, which is precisely the weighted percentage of these ingredients for the sunflower oil batch. Although weighted percentages and volume percentages are not the same measure, these results are rather interesting. The quantitative parameters found for the porosity, anisotropy and structure thickness illustrate how the use of sunflower oil results in a more stable and homogeneous protein network. Although the cooking loss is found to be greater in this sample, the POV for the entire water population of the sample (9.6%) is lower than for the lard sample (15.6%). This results has previously been shown in [Miklos et al. 2011], where the water separation was found to be 15.2% of the total sample weight when lard was used.

5 Conclusions

This paper has presented the use of a novel X-ray technique to measure the micro-structure of meat emulsions and the effect heat treatment and different lipid types have on the protein network. The grating-based technique obtained contrast levels superior to conventional X-ray absorption, making it possible to distinguish water from the protein mixture. For the analysis of the data, a segmentation method based on Gaussian mixture models and MRF labelling with graph cuts was implemented. Quantitative parameters were then extracted which represent the emulsion structure. These parameters include the POVs of the different ingredients and the porosity, degree of anisotropy and average structure thickness of the protein network. The results confirmed the difference in homogeneity of the protein network, which had already been inspected visually. Further analysis to the underlying interactions between the different lipids and protein network are out of the scope of this paper. Nevertheless, it has been shown that grating based X-ray imaging combined with multivariate contextual segmentation has enabled the investigation of changes in the 3D micro-structure of meat emulsions due to heat treatment.

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References

- Bech, M., Jensen, T.H., Bunk, O., Donath, T., David, C., Weitkamp, T., Le Duc, G., Bravin, A., Cloetens, P., Pfeiffer, F., 2010. Advanced contrast modalities for X-ray radiology: Phase-contrast and dark-field imaging using a grating interferometer. *Zeitschrift für Medizinische Physik*. 20, 7-16.
- Boykov, Y., Veksler, O., Zabih, R., 2001. Fast approximate energy minimization via graph cuts. *IEEE Trans. on Pattern Analysis and Machine Learning*. 23(11), 1222-1239
- Frisullo, P., Laverse, J., Marino, R., Nobile, M.A., 2009. X-ray computed tomography to study processed meat microstructure. *Journal of Food Engineering*. 94, 283-289.
- Hastie, T., Tibshirani, R., Friedman, J.H., 2009. *The elements of statistical learning*. Springer, 272-279
- Herremans, E., Chassagne-Berces, S., Chanvrier, H., Atoniuk, A., Kuszta, R., Bongaers, E., Verlinden, B.E., Jakubczyk, E., Estrade, P., Verboven, P., Nicolai, B., 2011. *Proceedings of InsideFood Workshop, Int.l Cong. on Engineering and Food*.
- Laverse, J., Frisullo, P., Conte, A., Nobile, M.A., 2012. X-ray microtomography for food quality analysis. *Food Industrial Processes – Methods and Equipment*, 339-362.
- Miklos, R., Xu, X., Lametsch, R., 2011. Application of pork fat diacylglycerols in meat emulsions. *Meat Science*, 87, 202-205.
- McDonald, S.A., Marone, F., Hintermuller, c., Mikuljan, G., David, C., Pfeiffer, F., Stampanoni, M., 2009. Advanced phase-contrast imaging using a grating interferometer. *Journal of Synchrotron Radiation*. 16, 562-572.
- Pfeiffer, F., 2012. Milestones and basic principles of grating-based X-ray and neutron phase-contrast imaging. *AIP Conference Proceedings*. 1466, 2-11.